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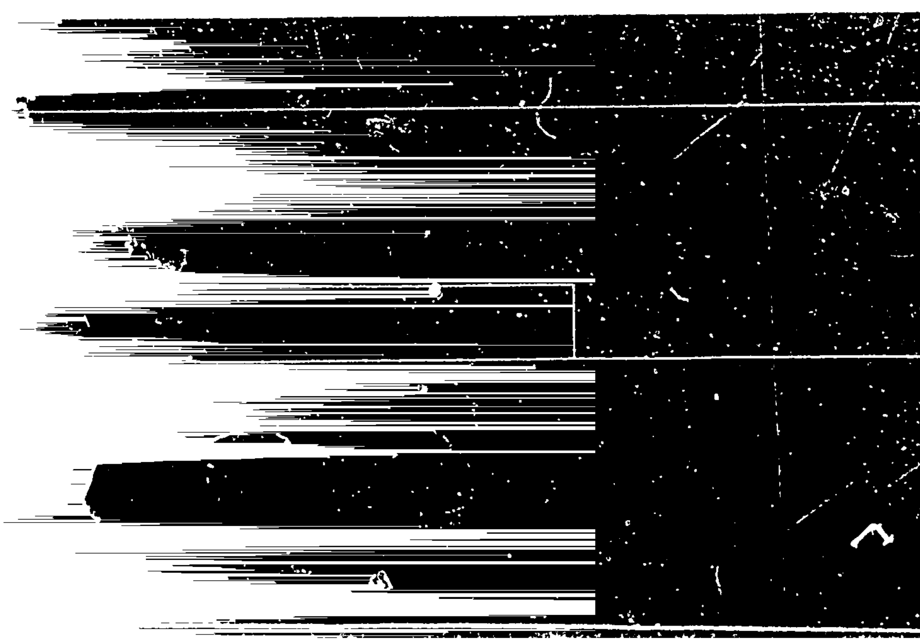

**COVER**

African Green monkey (*Cercopithecus aethiops*), small primate found throughout most of sub-Saharan Africa. This animal is now known to be naturally infected with a virus related to the AIDS virus of humans (HTLV-III/LAV), designated simian T-lymphotropic virus type III (STLV-$III_{AGM}$). It is postulated that the human AIDS virus in Africa may have arisen from this group of simian retroviruses. See page 951. [Stessen Casteel/FPG]

# Genomic Heterogeneity of AIDS Retroviral Isolates from North America and Zaire

Abstract. *In an analysis of the genomic variation of AIDS retroviral isolates from patients living in New York, Alabama, and Zaire, restriction maps were constructed by using seven enzymes, each known to cleave the proviral DNA more than once, in conjunction with Southern blot analysis. The maps of LAV, HTLV-III, and ARV-2 as deduced from their published nucleotide sequences were included in this analysis. The results demonstrated that (i) several "signature" restriction sites were common to all isolates; (ii) with the exception of LAV and HTLV-III, the North American and European isolates were all different from one another and showed no geographical specificity; (iii) the African isolates as a group were more diverse than those from North America and Europe; and (iv) the genomic variability was concentrated within the* env *gene.*

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The nucleotide sequences of three retrovirus (RV) isolates from patients with the acquired immune deficiency syndrome (AIDS) were recently published (*1–4*). A comparison of the *env* sequences indicated that the LAV and HTLV-III genomes differed from one another by 1.8 percent whereas LAV differed from the ARV-2 isolate by 9.3 percent (*5*). Structural heterogeneity of individual AIDS RV isolates, as monitored by restriction enzyme polymorphisms, had been observed previously (*6–8*); the extent of genomic variation was difficult to ascertain from such studies since selected autoradiograms of Southern blots, rather than detailed restriction enzyme maps, were published. The molecular cloning of different AIDS RV isolates followed by complete nucleotide sequencing would certainly provide definitive data regarding structural diversity. Operationally, such an approach would be both time consuming and expensive. Alternatively, one could construct a series of restriction maps using enzymes that cleave the proviral DNA at multiple sites. Although this latter approach is limited to the extent that restriction enzyme site polymorphisms may measure only single base-pair changes, nonetheless it can provide a rapid estimate of the degree of genomic variation between different AIDS RV isolates. Isolates with the same restriction maps may still differ in their DNA sequence.

In this study we examined the proviral DNA structure of North American and Zairian isolates of the AIDS RV. Peripheral blood lymphocytes (PBL) were obtained from five New York City patients in October 1984, from three Zairian patients in October 1983, and from a patient living in Birmingham, Alabama, in January 1985 (Table 1). Virus was isolated from the New York and Alabama patients by first stimulating their lymphocytes with phytohemagglutinin (PHA) and then propagating the cells in RPMI 1640 medium containing 10 percent fetal calf serum, five neutralizing units of antibody to α-interferon, and a 10 percent (by volume) solution of purified interleukin-2 (Electro-Nucleonics). The lymphocytes were monitored for the development of syncytia and supernatants were tested for reverse transcriptase (RT) activity. If no virus was isolated within 25 days, supernatants collected during this period were added to PHA-stimulated lymphocytes obtained from healthy donors and cultured for an additional 10 days as previously described (*9, 10*). Supernatant culture fluids from virus-producing cells were then used as inocula to infect PHA-stimulated normal lymphocytes (*10*) in the studies described below. The isolation of AIDS RV's from Zairian patients is described elsewhere (*11*).

Total cellular DNA was prepared as described (*12*) from the PHA-stimulated lymphocytes infected with different virus isolates, and cleaved to completion with seven restriction enzymes (Hind III, Bgl II, Sac I, Hinc II, Eco RI, Kpn I, and Nde I) known to cut AIDS proviral DNA at least twice. The DNA was then subjected to electrophoresis through 0.6 percent gels, transferred to nitrocellulose membranes, and analyzed by Southern blot hybridization (*13, 14*).

A molecular clone of an integrated LAV provirus was obtained by screening a λJ1 (*15*) phage library of virus-infected cellular DNA with a $^{32}$P-labeled complementary DNA (cDNA) probe (*16*). Of the $1.5 \times 10^6$ phage plaques surveyed (*17*) with the $^{32}$P-labeled LAV cDNA probe, three positive clones were obtained. One of these, containing integrated proviral DNA sequences extending from the 5′ long terminal repeat (LTR) to the Bam HI site at 8.4 kb (*18*) as well as 8.5 kb of 5′ flanking cellular DNA, was inserted

Table 1. Isolates of AIDS RV's from patients with AIDS or lymphadenopathy syndrome (LAS).

| Isolate | Sex | Disease | Date of isolation | Number of distinctive sites | Number of conserved sites |
|---|---|---|---|---|---|
| LAV | M | LAS | 1983 | 0 | 23 |
| HTLV-III* | M | LAS | 1983 | 0 | 23 |
| ARV-2 | M | LAS | 11/83 | 2 | 20 |
| NY-1 | M | LAS | 10/84 | 1 | 24 |
| NY-2 | M | LAS | 10/84 | 1 | 22 |
| NY-3 | M | AIDS | 10/84 | 3 | 21 |
| NY-4 | M | LAS | 10/84 | 0 | 21 |
| NY-5 | M | AIDS | 10/84 | 5 | 21 |
| Alabama | M | AIDS | 1/85 | 2 | 24 |
| Zaire-1 | M | AIDS† | 10/83 | 6 | 14 |
| Zaire-2 | F | AIDS | 10/83 | 5 | 14 |
| Zaire-3 | F | AIDS | 10/83 | 6 | 15 |

*Defined here as the virus present in the H9 line that was infected with multiple isolates collected in 1983 (*29*). †Suspected AIDS.

into the Bam HI site of pBR322 and designated pB2. Four contiguous viral DNA segments were subcloned into pBR322 as pB10, pB11, pB1, and pB4 (Fig. 1) and were radiolabeled with $^{32}$P-labeled deoxycytosine triphosphate to a specific activity of $5 \times 10^8$ to $10 \times 10^8$ cpm/μg by priming DNA synthesis with random hexamers (*19*).

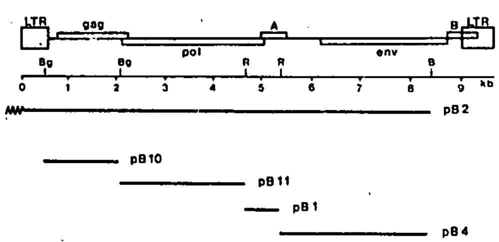
The restriction maps constructed are shown in Fig. 2. Also included are restriction maps of LAV, HTLV-III, and ARV proviral DNA's for the seven enzymes used as deduced from published Southern blots (*18*, *20*) or complete nucleotide sequences (*1–4*) of these isolates. In this analysis each isolate was treated as an independent entity; none was used as a prototype AIDS RV. If a restriction site was conserved in five or more isolates, it was indicated above the horizontal line representing each proviral DNA. Sites present in four or fewer isolates are shown below the line. Restriction sites unique to a particular isolate are enclosed within a "box."

With the exception of LAV and HTLV-III, all of the isolates were different (Fig. 2). However, some sites such as the Hind III sites in the LTR's, the Hinc II sites at 2.5 kb, the Kpn I site at 3.8 kb, and the Bgl II site at 7.0 kb were present in every isolate and could be considered a "signature" of the AIDS RV. In our analysis of AIDS RV genomic variation as monitored by digestion with seven enzymes, we focused on two characteristics: (i) the presence or absence of 24 "conserved" restriction sites in a particular isolate; (ii) the existence of novel sites in the proviral DNA. As shown in Table 1, the one French and eight North American isolates contained 20 to 24 of the 24 conserved restriction sites. In contrast, the three Zairian proviruses retained only 14, 14, and 15 of these sites, respectively. The African isolates, as a group, contained a greater number of distinctive restriction enzyme sites than the French/North American AIDS RV's. However, this difference was not as striking as the loss of conserved sites. For example, New York isolate No. 5 and Zairian isolate No. 2 both contained five novel restriction sites. A previous comparison of LAV and ARV-2 nucleotide sequences revealed significant heterogeneity in the envelope coding region (*5*). The differences observed consisted primarily of a series of small reciprocal insertions and deletions. When the location of distinctive restriction sites present in the different isolates were collectively analyzed, they were not randomly distributed throughout the viral genome but were concentrated in the *env* region (Table 2). To date we have observed no correlation between the number of conserved or distinctive sites and the replication efficiency of a particular isolate in PHA-stimulated lymphocytes. Similar amounts of virus are produced with NY-3 and Zaire-3 virus stocks despite the striking differences in the number of distinctive and conserved sites (Table 1).

Are the restriction enzyme polymorphisms observed with different AIDS RV isolates distinctive to this human T-lymphotropic virus or is genomic variation a common feature of mammalian retroviruses? Although their genomes have not been as extensively characterized as oth-

Table 2. Distribution of distinctive restriction sites in the AIDS RV genome. The LAV sequence reported by Wain-Hobson *et al.* (*1*) has been renumbered to conform with the format of a proviral DNA.

| Region | Proviral map position | Distinctive restriction sites |
|---|---|---|
| LTR | 1–638 | 1 |
| *gag* | 768–2294 | 3 |
| *pol* | 2087–5095 | 5 |
| A | 5009–5621 | 2 |
| Intergenic | 5622–6202 | 2 |
| *env* | 6203–8810 | 14 |
| B | 8781–9340 | 4 |
| LTR | 9098–9734 | 0 |



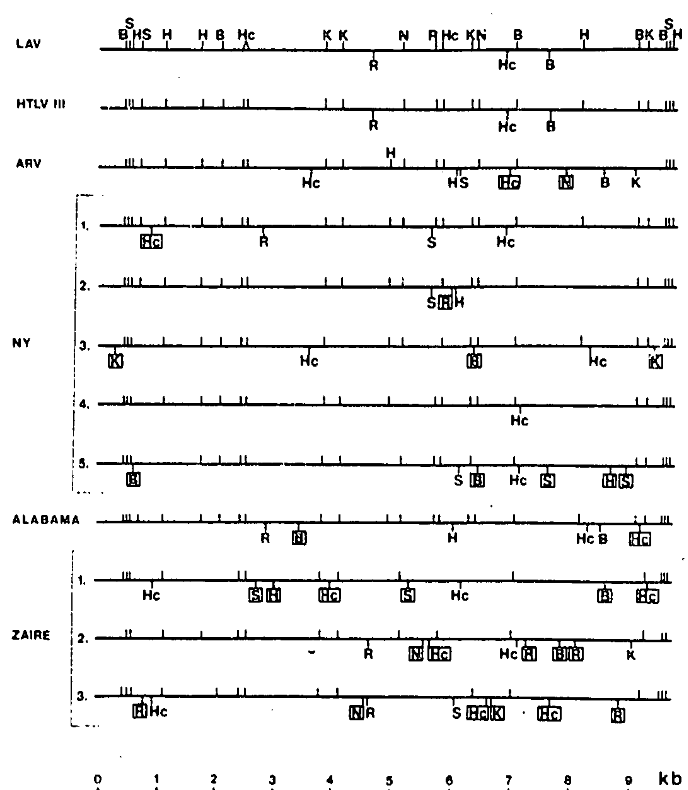




Fig. 1 (above). Locations of various recombinant plasmids relative to the LAV proviral DNA. The plasmid pB2 contains a 17-kb Bam HI insert consisting of 8.4 kb of viral DNA and 8.6 kb of 5′ flanking cellular DNA. The other recombinant plasmids were derived from pB2 after cleavage at the indicated sites and ligation to pBR322. Abbreviations: Bg, Bgl II; R, Eco RI; and B, Bam HI. Fig. 2 (right). Restriction endonuclease maps of AIDS RV proviral DNA's. Restriction sites present in different isolates were mapped by blot hybridization (*14*) of infected cellular DNA using the $^{32}$P-labeled subgenomic probes as shown in Fig. 1. The maps of LAV, HTLV-III, and ARV were derived from their published nucleotide sequences (*1–4*). The restriction maps are grouped according to the geographical origin of the isolates. A restriction site is indicated above the horizontal line when it is present in five or more isolates and below when it is found in four or fewer isolates. Distinctive sites are boxed. Abbreviations: B, Bgl II; S, Sac I; H, Hind III; Hc, Hinc II; K, Kpn I; R, Eco RI; and N, Nde I.

er retroviral subfamilies, antigenic variation involving the structure of the viral envelope is a characteristic feature of lentivirus isolates (*21–23*). Neutralization studies show that different antigenic strains of equine infectious anemia virus (EIAV), responsible for sequential febrile episodes, can be identified in individual diseased horses (*21*). These EIAV variants contain alterations confined to envelope glycoproteins as monitored by tryptic peptide mapping (*24*). Similar changes have been mapped to the *env* gene of visna virus antigenic variants (*25, 26*). Furthermore, molecular probes, respresentative of lentivirus genomes, do not hybridize to normal cellular DNA (*27*), indicating that these agents are exogenous, not endogenous retroviruses. The structural changes that appear in the viral genome are therefore not a consequence of recombination with related retroviral sequences that have been inserted into the germline of the infected cell.

These results clearly demonstrate the heterogeneity of AIDS RV genomes. Although the African isolates, as a group, were more diverse than those from North America, the U.S. samples were all different from one another and exhibited no geographical specificity. This conclusion differs, therefore, from that of Ratner *et al.* (*28*), who explain the genomic variation of HTLV-III and ARV on the basis of their New York and California origins, respectively. Since our analysis was based on restriction enzyme polymorphisms which, at best, measure single base changes, nucleotide sequencing of some of these new AIDS RV isolates will be required for a more complete assessment of the genomic relatedness. Such studies will be of importance in understanding how the AIDS RV exerts its immunopathogenic effects and in generating strategies for the development of new therapies and effective vaccines.



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## Isolation of T-Lymphotropic Retrovirus Related to HTLV-III/LAV from Wild-Caught African Green Monkeys

Abstract. *Present evidence suggests that the acquired immune deficiency syndrome (AIDS) emerged in Central Africa as a new disease in recent decades. This disease has recently approached epidemic proportions in many parts of the world. The etiologic agent of AIDS is believed to be the virus HTLV-III/LAV, which has been proposed as having originated from a recent simian-human transmission in Africa. This report describes the isolation of a designated STLV-III$_{AGM}$ retrovirus closely related to HTLV-III/LAV from seven healthy wild-caught African Green monkeys* (Cercopithecus aethiops) *that showed the presence of antibodies designated STLV-III$_{AGM}$. In vitro growth characteristics, ultrastructural morphology, and major proteins of 160,000 kilodaltons (kD), 120 kD, 55 kD, and 24 kD are similar to and cross-reactive with the analogous antigens of HTLV-III/LAV. The use of these serologic markers in the detection of STLV-III$_{AGM}$–infected monkeys may be important in assuring the continued safety of a variety of biologic reagents that are derived from these primate species. The existence of a retrovirus closely related to HTLV-III/LAV that naturally infects an African nonhuman primate in the apparent absence of disease may provide a unique model for the study of human AIDS and the development of an effective vaccine.*

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Studies on murine, feline, and bovine retroviruses have provided background and direction for much of the research on human retroviruses and the pathogenesis of leukemia and immunosuppression. The relevance of animal model systems is particularly exemplified by the recently described simian T-lymphotropic viruses (STLV), where present data on these exogenous type C retroviruses of nonhuman primates closely parallels observations of the biology of the human T-lymphotropic virus (HTLV) family. It is now widely recognized that a variety of Asian and African Old World primates are naturally infected with one or more retroviruses closely related to HTLV-I, designated STLV-I (*1*). In serologic studies, the association of STLV-I with spontaneous lymphoma in captive *Macaca* species has been noted paralleling the association of HTLV-I with adult T-cell leukemia/lymphoma (ATLL) in humans (*2*).

We recently described an exogenous retrovirus of captive ill macaques that is closely related to HTLV-III and designated it simian T-lymphotropic retrovirus type III (STLV-III$_{mac}$). The growth characteristics, T4 tropism, and ultrastructural morphology of STLV-III$_{mac}$